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THE COUNTRY CODE AND NUMBER OF YOUR PRIORITY APPLICATION, TO BE USED FOR FILING ABROAD UNDER THE PARIS CONVENTION, IS *US60/510,250*

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This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

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U.S. PTO 0074 30/510250

101003

INVENTOR(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Richard Agustin	Bond	Houston, Texas

☐ *Additional inventors are being named on the _____ separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

Method for treating airway diseases with beta-adrenergic inverse agonists

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☑ Specification *Number of Pages* 18

☑ Drawing(s) *Number of Sheets* 3

☐ Application Data Sheet. See 37 CFR 1.76

☐ CD(s), Number _____

☐ Other (specify) _____

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Respectfully submitted,

SIGNATURE _____

TYPED or PRINTED NAME Amie E. Franklin, Ph.D.

TELEPHONE (4150 341-4171

Date 10/10/2003

REGISTRATION NO. *(if appropriate)*

Docket Number: EGB001-PR

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Application Number	October 10, 2003
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First Named Inventor	Richard Agustin Bond
Examiner Name	
Art Unit	
Attorney Docket No.	EGB001-PR

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1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for *ex parte* reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
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1253	930	2253	465	Extension for reply within third month	
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1501	1,300	2501	650	Utility issue fee (or reissue)	
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SUBMITTED BY				(Complete *if applicable*)	
Name (Print/Type)	Amie E. Franklin, Ph.D.	Registration No. (Attorney/Agent)		Telephone	415-341-4171
Signature				Date	October 10, 2003

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Re: Method for treating airway diseases with beta-adrenergic inverse agonists

Our Docket No.: EGB001-PR

Dear Sir or Madam:

Enclosed please find the following documents related to the above-identified matter:

1. Provisional Application for Patent Cover Sheet (PTO/SB/16);
2. Fee Transmittal (PTO/SB/17);
3. Specification/claims;
4. Figures;
5. Self-addressed, stamped postcard.

The self-addressed, stamped postcard has been included for your convenience. After confirming receipt of these documents please return the postcard to us at your earliest convenience. Should you have any questions, please do not hesitate to contact me by phone at (415) 341-4171.

Sincerely yours,

Amie E. Franklin, Ph.D.

Enclosures

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Provisional Patent
Specifications/Claims

Docket No. EGB001-PR

Method for treating airway diseases with beta-adrenergic inverse agonists

Field of the Invention

The present invention relates to novel methods for preventing, treating, or reducing the severity of pulmonary airway diseases. In particular, it provides for methods and compositions for treating pulmonary airway diseases by long-term administration of beta adrenergic inverse agonist drugs. The present invention also contemplates administration of the beta adrenergic inverse agonist drugs in combination with beta 2 agonists, steroids, leukotriene modifiers, anticholinergics, adenosine receptor antagonists, phosphodiesterase-4 inhibitors, and anti-IgE antibodies.

Background of the Invention

Pulmonary airway diseases are characterized by reduced pulmonary function and airway flow often due to secretion of mucus, tissue inflammation, or tissue damage. These airway diseases include allergic rhinitis, asthma, cystic fibrosis, chronic obstructive pulmonary disease (COPD), Churg-Strauss syndrome, bronchitis, bronchiectasis, and emphysema.

Allergic rhinitis is essentially hay fever in which the upper airways of a human exhibit sensitivity to a large number of allergens, primarily pollens. This results in an inflammatory response of the airways due to an IgE-mediated response. The airways become infiltrated with eosinophils and other leukocytes. Additionally, there is marked tissue inflammation and mucus secretion which compromises airway function.

In cystic fibrosis, patients often exhibit increased airway resistance stemming from an inability to properly control epithelial chloride transport. One consequence is the presence of extremely viscous mucus in the airway. The mucus in the airway

predisposes the patient to pulmonary infections. Chronic infections of the lungs results in chronic inflammation of the airways leading to increased airway resistance.

COPD patients have obstructed airflow in the lungs. There are a number of ways that patients develop COPD, however the hallmark of the disorder is dyspnea, or breathlessness.

Churg-Strauss syndrome is an inflammatory disease in which patients exhibit asthmatic symptoms such as airway hyperreactivity. Inflammation of pulmonary airways occurs compromising pulmonary function.

In bronchitis, airway function is compromised due to hypersecretion of mucus initially due to irritants. With chronic bronchitis, coughing is persistent but may no longer be sufficient to clear airways leading to airflow obstruction.

Bronchietasis results from infection in the lungs leading to irreversible airway damage. Patients often complain of persistent cough and expectorate a foul-smelling sputum. Inflammation due to the infection in conjunction with the secretions contributes to airway obstruction despite the fact that bronchi and bronchioles can be exceptionally dilated.

Patients with emphysema have reduced pulmonary function due to destructive damage of the walls of lung alveoli. Often, patients are long-time smokers and have elevated levels of inflammatory cells such as neutrophils and macrophages in the lungs. The smoke is believed to activate lung neutrophils to release elastase, a damaging enzyme.

Asthma alone is a chronic problem for 17 million American patients. In asthma, patients exhibit airway hyperresponsiveness to minor provocations such as allergens, cold, and aspirin. These trigger immune system cells to release histamines, IgE molecules, cytokines or chemokines. Airway smooth muscle responds acutely to these provocations resulting in bronchial constriction. Additionally, the airway becomes damaged, inflamed, and mucous is secreted further limiting airway flow.

Patients with these airway disorders may have airway spasms, further reducing airflow through the pulmonary tree. During an attack, a patient's airway is constricted leading to difficulty breathing. Airway smooth muscle is responsible for the bronchoconstriction. The airway smooth muscle cells express beta-2 adrenergic receptors. Agonist binding to these receptors, such as epinephrine or beta-2 agonist drugs results in smooth muscle relaxation.

Consequently, for acute bronchospasms many patients inhale short-acting beta 2 adrenergic agonists which function to immediately relax smooth muscle of the airway. Alternatively, asthmatics may take long-acting beta-2 adrenergic agonists to prevent or reduce the severity of asthma attacks.

However, chronic administration of beta-adrenergic agonists has been demonstrated to lead to drug tolerance. Furthermore, there is also an increased hyperresponsiveness of the pulmonary airway in response to provocation such as allergans.

Epidemiological studies have demonstrated a positive correlation between the chronic use of short-acting beta-adrenergic agonists and asthma mortality. A large trial with the long-acting beta2-adrenergic agonist, salmeterol, was stopped due to increased death rates. This underscores that while short-term administration of beta agonists may be helpful to asthmatic patients, long-term administration may be deleterious.

Consequently, there is tremendous need for new therapeutic alternatives to beta 2 agonist use in asthmatics. Surprisingly, the inventor has discovered that chronic administration of beta adrenergic antagonists without partial agonist activity, termed inverse agonists, results in a lessoning of airway hyperreactivity.

Summary of the Invention

According to one aspect of the invention, a method of treatment is provided for pulmonary airway diseases by the administration of a therapeutically effective amount of a beta adrenergic antagonist drug with inverse agonist activity.

These inverse agonist drugs include beta 2 adrenergic antagonists without partial agonist activity and non-selective beta 1/beta 2 adrenergic antagonists without partial agonist activity.

The administration of beta antagonists has been contraindicated in patients with pulmonary airway hyperreactivity such as asthmatics and COPD patients. This is due to the observations that single-dose administration causes airway narrowing in asthmatic patients.

Beta antagonists were also once contraindicated for congestive heart failure (CHF). However, extensive clinical trials have repudiated this and now the beta antagonist carvedilol is approved by the FDA as a first-line therapy for CHF. Clinicians developed a very slow dosage ramping scheme to administer carvedilol safely to prevent any acute responses

Work carried out by the inventor has demonstrated that chronic administration of these antagonists results in reduced airway hyperresponsiveness.

According to another aspect of the invention, the therapeutic effect is a reduction in airway hyperresponsiveness.

The inventor has also demonstrated that administration of adrenergic inverse agonists results in a dramatic upregulation of the beta 2 adrenergic receptor. Chronic administration of carvedilol increases the level of the beta 2 adrenergic receptor as measured by radioligand measurements. Chronic administration of nadolol increases the level of the beta 2 adrenergic receptor as measured by immunohistochemistry.

According to another aspect of the invention, the therapeutic effect is an upregulation in the number of pulmonary beta 2 adrenergic receptors.

Additionally, chronic administration of these inverse agonists improves the relaxation responsiveness of the pulmonary airways to beta adrenergic agonists. This is in

contrast to chronic administration of beta adrenergic agonists which results in tolerance to these drugs and reduced effectiveness in ameliorating airway hyperresponsiveness.

It is well documented that chronic administration of beta adrenergic agonists cause agonist-dependent desensitization. Upon acute administration of beta agonists, adrenergic receptors are internalized thereby preventing them from being restimulated further for pulmonary relaxation. With chronic administration of beta agonists, there is actually a downregulation in the total number of beta adrenergic receptors. The consequence may be the observed loss of responsiveness seen in asthmatic patients on long-acting beta agonists.

According to another aspect of the invention, the therapeutic effect is to improve the pulmonary airway responsiveness to beta adrenergic agonists.

Detailed Description of the Invention

The use of cardioselective beta-antagonists (those with a preference for the beta 1 adrenergic receptor subtype) has been demonstrated to be safe in hypertensive and congestive heart failure (CHF) patients with chronic airway obstructive pulmonary disease (COPD).

Multiple studies have demonstrated that chronic administration of cardioselective beta antagonists does not change pulmonary function of CHF patients with COPD or asthma. Forced expiratory volume (FEV) was essentially unchanged in patients treated with cardioselective beta antagonists. These data indicate that chronic administration of cardioselective beta antagonists is safe in CHF patients with pulmonary airway disease. However, these drugs are ineffective in reducing or altering the symptoms of pulmonary airway disease.

D-propanolol has been proposed for the treatment of asthma (patents 5116867 and 6284800). The proposed target for D-propanolol is the adenosine receptor. Experiments performed (in patent 6284800) comparing the D versus the L forms of propanolol demonstrated that acute administration of the D form was beneficial in inhibiting

antigen-induced bronchoconstriction and reducing airway hyperresponsiveness. In contrast, acute administration of the L form increased specific lung resistance as expected for an active beta adrenergic antagonist. Normally, pharmaceutically-available propanolol is a racemic mixture of the D and L chiral forms. The D form of propanolol is inactive with respect to adrenergic receptors. Consequently, these two prior patents do not cover the inventor's claims of long-term administration of the active beta adrenergic binding form of propanolol, L-propanolol as part of a mixture, for the treatment of asthma.

Instead, this invention provides for the use of the active adrenergic receptor binding form of beta adrenergic antagonists that may be part of a racemic mixture.

According to another aspect of the invention, the beta adrenergic antagonists are non-selective beta1 and beta2 adrenergic antagonists without partial agonist activity or selective beta2 adrenergic antagonists without partial agonist activity. These drugs when administered chronically behave as inverse agonists.

Especially preferred for use according to the invention are nadolol (e.g. as HCl); bupranolol (e.g. as HCl); butoxamine (e.g. as HCl); carazolol (e.g. as HCl); carvedilol (e.g. as HCl); ICI 118551 (e.g. as HCl); levobunolol (e.g. as HCl); propanolol (e.g. as HCl); sotalol (e.g. as HCl); timolol(e.g. as HCl); and the salts, solvates, analogs, cogeners, hydrolysis products, metabolites, and precursors thereof.

Pharmaceutically acceptable salts include acid salts such as, hydrochloride, sulphate, fumarate, maleate, malonate, succinate, and tartrate, alkali metal salts such as sodium or potassium. Examples of solvates include hydrates.

The beta adrenergic inverse agonist may be used in conjunction with one or more pharmaceutical excipients.

Thus, the beta adrenergic inverse agonist may be formulated for oral, sustained-release oral, buccal, sublingual, inhalation, or parenteral administration.

Patients with pulmonary airway diseases often are prescribed multiple drugs that work in combination to control their symptoms.

According to the invention there is also provided for the administration of beta adrenergic inverse agonist in combination with beta2 selective adrenergic agonists for the treatment of pulmonary airway diseases.

The beta2 selective adrenergic agonists especially preferred for use according to the invention are albuterol, bitolterol, dobutamine, fenoterol, formoterol, levalbuterol, salbutamol, or salmeterol.

According to the invention there is also provided for the administration of beta adrenergic inverse agonist in combination with steroids.

The steroids especially preferred for use according to the invention are beclomethasone, budesonide, ciclesonide, flunisolide, fluticasone, methylpredisolone, prednisolone, prednisone, or triamcinolone.

According to the invention there is also provided for the administration of beta adrenergic inverse agonist in combination with anticholinergics.

The anticholinergics especially preferred for use according to the invention are ipratropium or tiotropium.

According to the invention there is also provided for the administration of beta adrenergic inverse agonist in combination with adenosine receptor antagonists.

The adenosine receptor antagonists especially preferred for use according to the invention are theophylline, extended-release theophylline, theobromine, or caffeine.

According to the invention there is also provided for the administration of beta adrenergic inverse agonist in combination with an anti-IgE antibody.

The anti-IgE antibody especially preferred for use according to the invention is omalizumab.

According to the invention there is also provided for the administration of beta adrenergic inverse agonist in combination with leukotriene modifiers.

The leukotriene modifiers especially preferred for use according to the invention are ibudilast, montelukast, pranlukast, and zafirlukast.

According to the invention there is also provided for the administration of beta adrenergic inverse agonist in combination with a phosphodiesterase-4 inhibitor.

The phosphodiesterase-4 inhibitors especially preferred for use according to the invention are roflumilast or cilomilast.

EXAMPLES

EXAMPLE 1

Airway resistance reduction by chronic administration of beta adrenergic inverse agonists

Methods

Balb/cJ mice aged 6 weeks (Jackson Animal Laboratory, Bar Harbor, Maine) were housed under specific pathogen-free conditions and fed a chicken ovalbumin-free diet. The Animal Research Ethics Boards of both the University of Houston and Baylor College of Medicine approved all experiments reported here. The effects of administration of the non-selective beta antagonists carvedilol (GlaxoSmithKline, King of Prussia, PN), nadolol (Sigma Chemical, St. Louis, MO), and of salbutamol (Sigma Chemical, St. Louis, MO), a beta2 adrenergic partial agonist, were examined in a murine model that exhibited cardinal features of human asthma, such as pulmonary

eosinophilic inflammation, airway hyperresponsiveness, and heterogenous airway narrowing. The results obtained in drug-treated animals were compared with those obtained in vehicle-treated counterparts in experiments performed in close temporal relationship. The outcome measures of this study included statistically-significant differences between drug-treated mice and non-treated animals in terms of baseline airway resistance, degree of airway responsiveness to cholinergic stimulation, and bronchoalveolar lavage (BALF) cellularity. Mice were sensitized with subcutaneous injection of 25 μg of ovalbumin adsorbed to aluminum hydroxide on protocol days 2, 9, and 16. Subsequently, mice were given 50 μl of saline solution containing 25 μg of ovalbumin intranasally, on a daily basis, from protocol days 23 through 27. A group of ovalbumin-sensitized saline-challenged mice served as controls for systemic sensitization and respiratory challenges with ovalbumin. Prior to intranasal administrations, mice were sedated with halothane vapor. For this study, ovalbumin-sensitized and ovalbumin-challenged mice, and ovalbumin-sensitized and saline-challenged mice will be referred to as asthmatic mice and control mice, respectively. The drugs used were salbutamol (a beta2 adrenergic partial agonist), aprenolol (a beta1/beta2 adrenergic antagonist with partial beta2 agonist activity), nadolol and carvedilol (both nonselective beta1/beta2 adrenergic antagonists with inverse agonist activity at the beta2 adrenergic receptor).

To examine the effects of duration of beta adrenergic ligand therapy on the phenotype of the murine model of asthma, the experimental drugs were administered either acutely or chronically to different groups of asthmatic mice.

Asthmatic mice on acute therapy were given a single intravenous bolus infusion of either beta adrenergic drug or normal saline on protocol day 28, 15 minutes before airway responsiveness to methacholine was determined. The doses of carvedilol, nadolol, aprenolol, and salbutamol administered to the mice were 24 mg/kg, 72 mg/kg, 72 mg/kg, and 0.15 mg/kg, respectively. Asthmatic mice on chronic therapy were treated with the beta adrenergic drug during protocol days 1 to 28. Those on beta antagonists had free access to chow treated with carvedilol, nadolol, or alprenolol at

concentrations of 2400 ppm, 250 ppm, or 7200 ppm, respectively. These concentrations were chosen based on those producing therapeutic effects in mice in previously published studies. The non-treated asthmatic mice were fed normal chow. Salbutamol was delivered for 28 days at a dose of 0.5 mg/kg/day using an osmotic minipump (Alzet®, #2004, Durect Corporation, Cupertino, CA).

On protocol day 28, mice were anesthetized, tracheotomized, and connected to a computer-controlled small animal ventilator (Flexivent, Scientific Respiratory Equipment, Inc., Montreal, Canada). Airway resistance (R_{aw}) was measured using the forced oscillation technique. The cellular composition of bronchoalveolar lavage fluid (BALF) was also determined. In non-treated asthmatic mice, the degree of airway responsiveness and the number of eosinophils recovered in BALF were significantly higher compared to the ovalbumin-sensitized saline-challenged (control) mice. However, we observed that the degree of airway responsiveness and the number of eosinophils recovered in BALF were lower in non-treated asthmatic mice studied in close temporal relationship with mice receiving acute beta adrenergic antagonist treatments than in those obtained in non-treated asthmatic mice studied concomitantly with mice on chronic beta adrenergic antagonist therapy.

To induce airway constriction, a solution containing 150 μg/ml of acetyl-α-methylcholine chloride (methacholine) (Sigma Chemical, St. Louis, MO) was infused intravenously at constant rates using a syringe infusion pump (Raze Scientific Instruments, Stanford, CN). The methacholine infusion was started at 0.008 ml/min, and its rate was doubled stepwise up to a maximum of 0.136 ml/min. Each methacholine dose was administered for 3 to 5 minutes, during which data were sampled at 1 minute intervals and then averaged.

Data Analysis

The complex input impedance of the respiratory system was computed and the value of the real part of respiratory system impedance at 19.75 Hz was taken to reflect the magnitude of airway resistance (R_{aw}). To examine the degree of airway responsiveness of each animal, the values for R_{aw} as a function of methacholine doses were plotted.

The largest value for R_{aw} obtained in response to methacholine stimulation was referred to as R_{awpeak}. For mice that achieved a plateau in the methacholine dose-R_{aw} response curve, the ED_{50} was calculated by linear interpolation using the GraphPad Prism4 (GraphPad Software, Inc.). Results were expressed as mean±SEM. Comparisons between results obtained for beta adrenergic drug treated and non-treated mice were performed using the analysis of variance for multiple groups of a student's t-test for comparing two groups. The Bonferroni test was used to examine the statistical differences between experimental groups. The effects of acute drug treatments on baseline respiratory system mechanics were assessed using two-tailed paired t-test. A value of $P<0.05$ was considered statistically significant.

Figure 1

Figures 1A and 1B shows that methacholine provocation significantly enhances airway resistance (R_{aw}) in asthmatic mice in contrast to a minimal response upon saline provocation of asthmatic mice. This demonstrates that the mouse model in this study exhibits airway hyperresponsiveness, a key feature of airway dysfunction in human asthma.

In Figure 1C, the administration of a single intravenous bolus of salbutamol to asthmatic mice reduced the level of airway responsiveness to methacholine provocation and the level of airway resistance as expected. In Figure 1D when salbutamol was delivered for 28 days to the mice, no protection was observed. This lack of reduction of airway hyperresponsiveness upon chronic administration of a beta adrenergic agonist has been observed in humans when tolerance to these drugs develop.

In Figure 1E, when asthmatic mice were given a single intravenous bolus of alprenolol, a beta adrenergic antagonist with partial agonist activity, their airway responsiveness was diminished, as indicated by significant decreases in both the values for R_{aw} at methacholine doses ≥408 μg/kg/min. ($P<0.05$) compared to those obtained in non-treated counterparts. The reduction in airway responsiveness upon acute administration of alprenolol is similar to that observed for salbutamol, consistent with the partial agonist activity that alprenolol possesses. In Figure 1F, when asthmatic mice were exposed to

alprenolol for 28 days, their average methacholine dose-response relationship was similar to that obtained in nontreated mice demonstrating that provides no benefit upon chronic administration as is the case with salbutamol.

In Figure 1G, a single intravenous bolus of carvedilol enhanced the airway responsiveness in the asthmatic mice. This is consistent to previous observations in humans that acute delivery of beta adrenergic antagonists to asthmatics can result in severe airway constriction. In contrast, in Figure 1H, chronic administration of carvedilol reduced the responsiveness of asthmatic mice to methacholine provocation. Chronic delivery of carvedilol not only decreased the airway constrictor response at high doses of methacholine, it also shifted the methacholine dose-airway response relationship to the left of that obtained in the non-treated asthmatic mice.

In Figure 1I, a single intravenous bolus of nadolol also enhanced the airway responsiveness of asthmatic mice similar to that observed for carvedilol. Chronic delivery of nadolol shown in Figure 1J also produced a decrease in airway responsiveness, which was more pronounced that that caused by long-term carvedilol treatment. Indeed, the average methacholine dose-R_{aw} response relationship obtained in asthmatic mice on chronic nadolol treatment was similar to that obtained in mice on acute salbutamol treatment.

Example 2

Chronic inverse agonist treatment increases beta adrenergic receptor numbers as measured by radioligand binding.

Beta 2 adrenergic receptor numbers were measured non-drug-treated asthmatic mice and in asthmatic mice chronically-treated with the beta adrenergic inverse agonist, carvedilol and the beta adrenergic antagonist, alprenolol. Mice were sacrificed and lung membranes were isolated as follows. Frozen lung tissue was homogenized in an ice-cold buffer containing 0.32M sucrose and 25mM Tris (pH 7.4) using a polytron (Pro 200, Pro Scientific, Inc.). The homogenate was centrifuged at 1000 *g* for 10 min at 4°C. This supernatant was centrifuged at 40,000 *g* for 20 min at 4°C. The pellet was suspended in

an ice-cold 25 mM Tris-HCl buffer (pH 7.4) and centrifuged at 40000 *g* for 20 min at 4°C. The final pellet was suspended in 200µl 25mM Tris-HCl (pH 7.4), membrane protein concentration was determined by BCA protein assay kit. Radioligand receptor binding incubation mixtures contain membranes (~10 µg of protein), (-)3-[125I]-cyanopindolol (ICYP) in 25mM Tris-HCl, pH 7.4) in increasing concentrations (5–7500 pM) and binding buffer in a final volume of 250 µl. Propranolol was used to determine nonspecific binding. The incubation was done at 37°C for 2 h and terminated by rapid vacuum filtration through glass fiber filters. The filters were washed three times with 250 µl of ice cold wash buffer (25 mM Tris-HCl, pH 7.4) and the radioactivity determined in a counter. All experiments were performed in triplicate and receptor densities are expressed as picomoles of sites per milligram of protein. Bmax is determined by nonlinear regression of the saturation binding curves. Data are the means ± S .E.M (n=3). (□) P <0.001 compared to other groups (ANOVA, Bonferoni correction).

Radioligand binding revealed that beta2 adrenergic receptor levels are not altered merely by the absence or presence of methacholine challenge as seen by the essentially similar levels of beta 2 adrenergic receptors in both the methacholine-challenged and the unchallenged non-drug treated asthmatic mice as shown in Table 1. Chronic alprenolol treatment led to a slight doubling of the level of the beta2 adrenergic receptor. Most significantly, was the over 10-fold increase of beta2 adrenergic receptors in the carvedilol-treated mice over the non-treated mice, demonstrating the efficacy of this beta adrenergic inverse agonist in increasing receptor levels upon chronic administration.

Example 3

Chronic inverse agonist treatment increases beta adrenergic receptor numbers as monitored by immunohistochemistry.

For immunohistochemistry analysis of beta2 adrenergic receptor levels, non-drug-treated control mice and mice treated chronically with the beta adrenergic inverse agonist nadolol were used. The mice were sacrificed and the lungs excised. Then the lungs were fixed in 4% paraformaldehyde (45 min, 0°C). After fixation, lungs were

washed in PBS (60 min) and placed in increasing concentrations of sucrose (10% sucrose/5% glycine in PBS for 30 min; 20% sucrose/10% glycine in PBS for 30 min; 30% sucrose/15% glycine in PBS for 12 h at 4oC). Lungs were embedded in OCT and 12µm sections cut with a Tissue-Tek II cryostat. The sections were air dried and fixed with 4% paraformaldehyde for 15 min. After 3 washes in PBS, the slides were blocked with 5% milk in PBS for 1 h, and then incubated overnight with anti-beta2 adrenergic receptor antibody (1:200; Santa Cruz Biotechnology) in blocking solution. Slides were washed in PBS and incubated with secondary antibody (1:200; Cy3-goat anti-rabbit, 16 h at 4oC). Control slides were incubated with antibody specific blocking peptide to demonstrate specificity of binding of the primary antibody. After washing with PBS, coverslips were mounted and viewed by epifluorescent microscopy.

As shown in figure 2, labeling with anti-beta2 adrenergic receptor antibodies was considerably more intense in lungs from treated animals than in lungs from animals not treated with nadolol. Loss of this signaling upon incubation in the presence of the beta2 adrenergic receptor peptide, demonstrates that this antibody is specifically binding the beta2 adrenergic receptor. This observation is consistent with the radioligand binding data and suggests beta2 adrenergic receptors are effectively upregulated by chronic administration of beta adrenergic inverse agonist drugs.

Claims:

1. A method for treatment of a pulmonary airway disease in a human by the administration of a therapeutically effective amount of a beta adrenergic antagonist drug with inverse agonist activity.
2. The method of claim 1, wherein the therapeutic effect is a reduction in pulmonary airway constriction hyperresponsiveness.
3. The method of claim 1, wherein the therapeutic effect is an upregulation in pulmonary beta 2 adrenergic receptors.
4. The method of claim 1, wherein the therapeutic effect is increased pulmonary airway relaxation responsiveness to beta 2 adrenergic agonist drugs.
5. The method of claim 1, wherein the beta-adrenergic inverse agonist is administered in an oral, a sustained-release oral, parenteral, sublingual, buccal, or inhalation dosage form.
6. The method of claim, 1 wherein the patient has a pulmonary airway disease.
7. The method of claim 6, wherein the patient has asthma, allergic rhinitis, bronchiectasis, bronchitis, chronic obstructive pulmonary disease (COPD), Churg-Strauss syndrome, cystic fibrosis, emphysema, or pneumonia.
8. The method of treatment of claim 1, wherein the beta adrenergic antagonist drug with inverse agonist activity is selected from the group consisting of nadolol, bupranolol, butoxamine, carazolol, carvedilol, ICI 118551, levobunolol, propanolol, sotalol, timolol, or the analogs or cogeners of these drugs.
9. The method of treatment of claim 1, wherein the method of administration of the drug results in continuous levels of the drug in the patients bloodstream.
10. A method for treatment of pulmonary airway diseases in a human by the administration of therapeutically effective beta adrenergic inverse agonist drug in combination with a beta-2 selective adrenergic agonist drug.
11. The method of treatment of claim 10, wherein the beta-2 selective adrenergic agonist drug is selected from the group consisting of albuterol, bitolterol, dobutamine, fenoterol, formoterol, levalbuterol, pirbuterol, salbutamol, salmeterol, or terbutaline.

12. A method for treatment of pulmonary airway diseases in a human by the administration of therapeutically effective beta adrenergic inverse agonist drug in combination with steroids.
13. The method of treatment of claim 12, wherein the steroid is selected from the group consisting of beclomethasone, budesonide, ciclesonide, flunisolide, fluticasone, methylpredisolone, prednisolone, prednisone, or triamcinolone.
14. A method for treatment of pulmonary airway diseases in a human by the administration of therapeutically effective beta adrenergic inverse agonist drug in combination with an anticholinergic drug.
15. The method of treatment of claim 14, wherein the anticholinergic is selected from the group consisting of ipratropium or tiotropium.
16. A method for treatment of pulmonary airway diseases in a human by the administration of therapeutically effective beta adrenergic inverse agonist drug in combination with an adenosine receptor antagonist.
17. The method of treatment of claim 16, wherein the adenosine receptor antagonist is selected from the group consisting of theophylline, theobromine or caffeine.
18. A method for treatment of pulmonary airway diseases in a human by the administration of therapeutically effective beta adrenergic inverse agonist drug in combination with an anti-IgE antibody.
19. The method of treatment of claim 18, wherein the anti-IgE antibody is omalizumab.
20. A method for treatment of pulmonary airway diseases in a human by the administration of therapeutically effective beta adrenergic inverse agonist drug in combination with leukotriene modifiers.
21. The method of treatment of claim 20, wherein the leukotriene modifier is selected from the group consisting of ibudilast, montelukast, pranlukast, and zafirlukast.
22. A method for treatment of pulmonary airway diseases in a human by the administration of therapeutically effective beta adrenergic inverse agonist drug in combination with a phosphodiesterase-4 inhibitor.

23. The method of treatment of claim 22, wherein the phosphodiesterase-4 inhibitor is selected from the group consisting of roflumilast or cilomilast.

Figure 1.

The effects of treatments with beta adrenergic drugs on airway responsiveness to methacholine in a murine model of asthma.

Asthmatic mice received either a single intravenous bolus injection 15 minutes prior to methacholine challenge (acute; top row) or were treated for 28 days (chronic; bottom row). Average methacholine dose-airway resistance relationships were obtained in control mice (Ctrl ○, N = 6-21), non-treated asthmatic mice (NTX ●, N = 7-25), and in asthmatic mice treated with the beta adrenergic drugs (□, N = 8-19). Values are mean ±SEM. Please note the change in the scale of the y-axis for panels G and I

Panels A and B: no drug treatment, control mice and non-treated asthmatic mice

Panels C and D: salbutamol treatment

Panels E and F: alprenolol treatment

Panels G and H: carvedilol treatment

Panels I and J: nadolol treatment

Table 1. Chronic inverse agonist treatment increases beta adrenergic receptor numbers as measured by radioligand binding.

Asthmatic mice (ovalbumin-sensitized) were treated as follows: no drug treatment and no methacholine challenge, no drug treatment with methacholine challenge, chronic carvedilol treatment with methacholine challenge, and chronic alprenolol treatment with methacholine challenge. The receptor number is provided as a concentration, K_D, given in pM, picomolar ± SEM. Bmax is given as pmol/mg ± SEM.

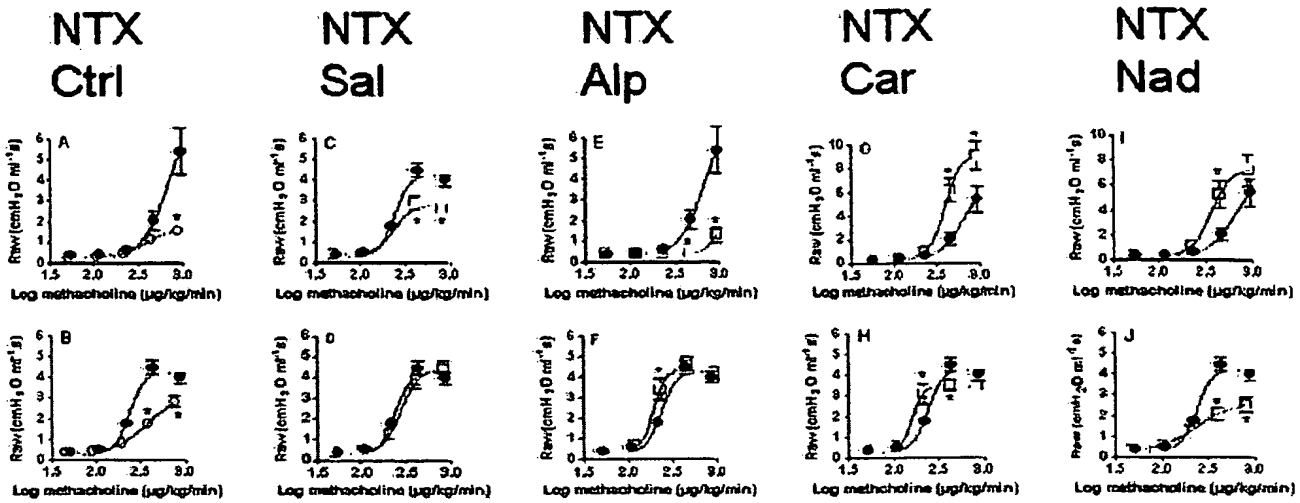
Figure 2. Chronic inverse agonist treatment increases beta 2 adrenergic receptor numbers as measured by immunohistochemistry.

Lung sections from non-drug-treated mice and from chronically-treated nadolol mice were stained with anti-beta2 adrenergic receptor antibodies in the presence and absence of competing beta2 adrenergic receptor peptide. In panel A, very little staining is present in the non-drug-treated mice whereas in panel C, the nadolol-treated mice had a significant level of staining. In panels B and D, addition of the competing peptide eliminated all signals demonstrating that the original signals were due to the presence of beta 2 adrenergic receptors.

Provisional Patent

Docket No. EGB001-PR

Drawings



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Treatment	KD (pM)	Bmax (pmol/mg)
No drug sensitized only	131.3 ± 85.8	0.258 ± 0.065
No drug sensitized, challenged	110.2 ± 37.9	0.245 ± 0.026
Carvedilol sensitized, challenged	*1484.0 ± 64.8	*0.965 ± 0.032
Alprenolol sensitized, challenged	205.6 ± 55.7	0.156 ± 0.052

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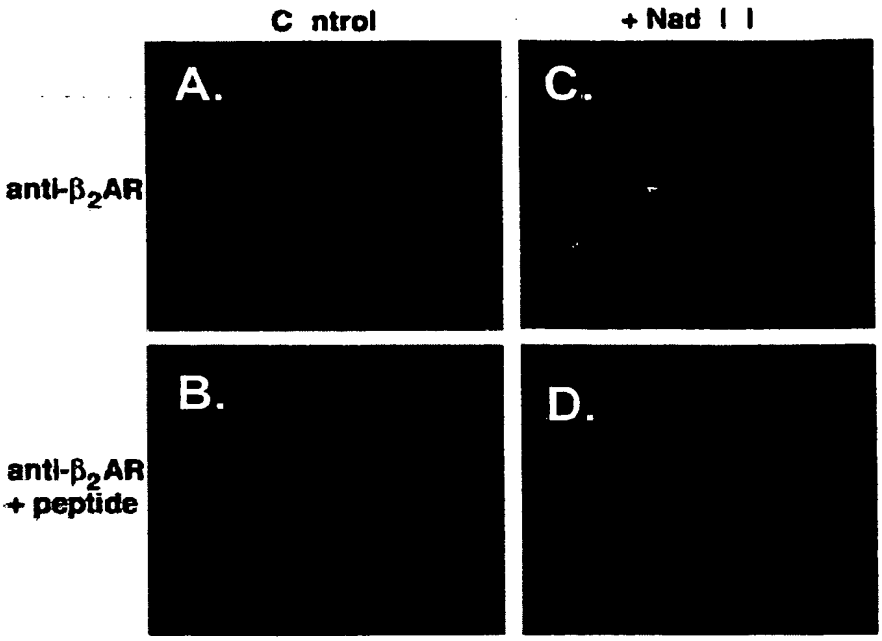

Figure 2. Chronic inverse agonist treatment increases beta 2 adrenergic receptor numbers as measured by immunohistochemistry.

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